# THE EFFECT OF ALKALINITY UPON MUTUAL INFLU-ENCES DETERMINING THE DEVELOP-MENTAL AXIS IN FUCUS EGGS <sup>1</sup>

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## INTRODUCTION

When eggs of *Fucus furcatus* develop in the dark in normal sea water in masses they tend to form rhizoids in the resultant direction of neighboring eggs (Whitaker, 1937). This phenomenon, which has been referred to as the "group effect," does not occur when only two eggs are present in a dish unless the medium is acidified. Thus, it has been reported earlier (Whitaker, 1937) that of about 200 eggs reared in pairs (at distances of 4 egg diameters or less) in sea water acidified to pH 6.0,<sup>2</sup> approximately 90 per cent formed rhizoids on the sides toward the neighbor, while in normal sea water at pH 7.8–8.0 only 41 per cent of about 130 eggs did so. Acidification of the medium thus strongly intensifies the "group effect."

It is of some importance in the study of the phenomenon to know whether the strong attraction which exists between two eggs in acid sea water is merely abolished at higher pH, so that each egg is unaffected by the presence of a neighbor, or whether an actual repulsion, or negative "group effect," exists. The fact that less than 50 per cent formed rhizoids toward neighbors at pH 7.8–8.0, in the count repeated above, suggests a negative "group effect," but the numbers are inadequate. Experiments have therefore been undertaken to test this point, extending the pH range as far in the basic direction as possible without much alteration of the composition of the sea water, and with special attention to exactness in pH determination.

#### Method

Material was obtained and treated in a manner previously described (Whitaker, 1936). Experiments were carried out during March, 1938. The eggs were kept in the dark, or in dim red light, in a constant tem-

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 $<sup>^2</sup>$  Acidified either with citric acid-secondary sodium phosphate, or with HCl–NaHCO\_{\tiny 3}.

perature room at  $15 \pm \frac{1}{4}^{\circ}$  C., from the time of shedding until the results were recorded. The eggs were shed and were fertilized in normal sea water, and were transferred to sea water of artificially altered pH 30-60 minutes after fertilization.

## TABLE I

Conditions of the experiments. Eggs were reared two in a dish at distances of 0.3-4 egg diameters, in the dark at  $15 \pm \frac{1}{4}$ ° C. Experiments are arbitrarily numbered in order of pH sequence. The symbol S.W. in column 2 refers to sea water. The pH difference in column 5 shows the change in pH of the medium during the experiment. The results of these experiments are presented graphically in Fig. 1.

Exp. No.	Medium				Total	Rhizoids
	Composition	pH at start	pH after 24 hrs.	pH change in 24 hrs.	No. Eggs	toward Neighbor per cent
1	S.W. + KOH	8.85	8,68	-0.17	88	42
2	$S.W. + Na_2CO_3 - NaHCO_3$	8.81	8.72	-0.09	74	39
3	S.W. + KOH	8.61	8.48	-0.13	84	29
4	S.W. $+$ Na <sub>2</sub> CO <sub>3</sub> $-$ NaHCO <sub>3</sub>	8.58	8.53	-0.05	69	32
5	S.W. $+$ Na <sub>2</sub> CO <sub>3</sub> $-$ NaHCO <sub>3</sub>	8.40	8.32	-0.08	119	27
6	S.W. $+$ Na <sub>2</sub> CO <sub>3</sub> $-$ NaHCO <sub>3</sub>	8.41	8.36	-0.05	30	23
7	S.W. + KOH	8.42	8.30	-0.12	26	19
8	$S.W. + Na_2CO_3 - NaHCO_3$	8.41	8.35	-0.06	18	22
9	$S.W. + Na_2CO_3 - NaHCO_3$	8.43	8.34	-0.09	36	22
10	$S.W. + Na_2CO_3 - NaHCO_3$	8.22	8.17	-0.05	81	22
11	$S.W. + Na_2CO_3 - NaHCO_3$	8.22	8.15	-0.07	68	28
12	$S.W. + Na_2CO_3 - NaHCO_3$	8.00	8.12	+0.12	94	38
13	S.W.	8.01	7.93	-0.08	21	38
14	S.W.	8.01	8.09	+0.08	14	36
15	S.W.	7.80	7.70	-0.10	87	37
16	S.W. + citric acid	7.80	7.73	-0.07	52	42
17	S.W. + citric acid	7.61	7.67	+0.06	63	-48
18	S.W. + citric acid	7.59	7.65	+0.06	42	52
19	S.W. + citric acid	7.62	7.65	+0.03	38	47
20	S.W. + citric acid	7.58	7.63	+0.05	22	45
21	S.W. + citric acid	7.40	7.43	+0.03	85	56
22	S.W. + citric acid	7.41	7.37	-0.04	32	59
23	S.W. + citric acid	7.41	7.46	+0.05	22	59
24	S.W. + citric acid	7.20	7.27	+0.07	46	76
25	S.W. + citric acid	7.22	7.21	-0.01	60	68
26	S.W. $+$ citric acid	7.21	7.25	+0.04	24	71
27	S.W. + citric acid	6.99	7.10	+0.11	22	86
28	S.W. + citric acid	7.00	7.11	+0.11	62	76
29	S.W. + citric acid	7.02	7.08	+0.06	51	80

The pH of filtered sea water, which was collected at frequent intervals, ranged from 7.8–8.0. Samples were made more basic by adding either KOH, or a mixture of  $Na_2CO_3$  and  $NaHCO_3$ . Other samples were acidified by adding citric acid. In all cases the osmotic pressure was maintained constant at the time of these additions, and the mixture was vigorously aerated with a large scintered glass nozzle to reequilibrate with atmospheric  $CO_2$  tension. A glass electrode was used to measure pH, and the aeration was continued until a constant and stable pH was attained. The pH range covered was from 7.0 to 8.8 at intervals of 0.2, as closely as could be approximated. In each experiment pH was measured at the beginning, and also at the end 24 hours later. These pH values, and the composition of the media, are shown in Table I. While the glass electrode was not accurate to the second decimal of pH determination, it approached this accuracy, and therefore the values are recorded to the second decimal as read.

Sea water was not alkalinized much above pH 8.8 because of precipitation of certain salts. There is slight precipitation even at pH 8.8, and at 8.6. The development is altered in the precipitation range so that the rhizoid becomes small and very narrow at the base (Whitaker and Lowrance, 1937) although whether this is due to pH or to loss of salts from the medium is not known.

Eggs were transferred individually into experimental medium in 1 cc. Syracuse dishes by means of a very small pipette. The pipette contained experimental medium which was caused to flow about the egg before it was taken up, and then less than 0.01 cc. solution was carried over with it. Two eggs were placed in each dish at a distance from each other of 0.3–4 egg diameters. The eggs are  $65–90 \,\mu$  in diameter. The dishes were already mounted in moist chambers on a level platform on a vibrationless concrete table.

## **RESULTS AND CONCLUSIONS**

The results are shown graphically in Fig. 1. Each point in Fig. 1 represents the results of one experiment, and conditions of each experiment are given in Table I.

It is clear from Fig. 1 that a negative "group effect" exists above pH 7.6. This appears to reach a maximum at pH 8.4, when approximately 80 per cent of the eggs form rhizoids on the sides (halves) away from the neighbor. Above pH 8.4 this percentage declines, but since the medium is changing due to precipitation, and the development of the rhizoid is altered, it is not certain that the results above pH 8.4 are entirely comparable. For this reason the curve is dotted in Fig. 1. This evidence of negative "group effect" between 2 eggs in the higher pH range is in agreement with the response of single eggs developing in the ends of capillary tubes in their own diffusion gradients (Whitaker and Lowrance, 1937).

In Table I and Fig. 1 the results are grouped by experiments and pH, without attention to distance between eggs, within the limits 0.3–4 egg diameters. The effect of distance may be seen by dividing the results at a given pH into categories on the basis of distance between eggs.



FIG. 1. Results of experiments defined in Table I, showing reversal from positive to negative "group effect" with increasing pH. Each circle represents the results of one experiment, and shows the percentage of eggs which formed rhizoids on the half toward the neighbor. The experiments are grouped at intervals of approximately 0.2 pH unit, with 129–229 eggs at each interval. The curve is dotted through pH 8.6 and 8.8 because of alteration of the medium by precipitation (see text).

At pH 7.0, the percentages of eggs forming rhizoids on the halves toward the neighbor, at different distances, are as follows: 0.3–1 egg diameter, 90 per cent; 1–2 diameters, 75 per cent; 2–3 diameters, 72 per cent; 3–4 diameters, 70 per cent. At pH 8.4 the corresponding figures are: 0.3–1 diameter, 21 per cent; 1–2 diameters, 21 per cent; 2–3 diameters, 31 per cent; 3–4 diameters, 36 per cent. It is seen that within these limits the effect is stronger the closer the eggs, in the pH ranges of both positive and negative "group effect."

## SUMMARY

1. When two eggs of *Fucus furcatus* develop in the dark within 0.3–4 egg diameters of each other, the point of rhizoid origin and the developmental axis are influenced by the presence of the neighbor.

2. When the pH of the medium is below approximately 7.6, the rhizoids tend to form on the sides of the eggs toward the neighbor. When it is above 7.6, they tend to form on the sides away from the neighbor, most markedly at pH 8.4.

3. The effect is stronger the closer the eggs, within the limits 0.3–4 egg diameters.

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